

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference INECL/P27397PC	FOR FURTHER ACTION	
		See Form PCT/IPEA/416
International application No. PCT/GB2004/001180	International filing date (day/month/year) 18.03.2004	Priority date (day/month/year) 19.03.2003
International Patent Classification (IPC) or national classification and IPC C12P41/00		
Applicant INEOS FLUOR HOLDINGS LIMITED et al.		

<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 12 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input checked="" type="checkbox"/> (<i>sent to the applicant and to the International Bureau</i>) a total of 2 sheets, as follows:</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions). <input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box. <p>b. <input type="checkbox"/> (<i>sent to the International Bureau only</i>) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>
<p>4. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> Box No. I Basis of the opinion <input type="checkbox"/> Box No. II Priority <input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability <input type="checkbox"/> Box No. IV Lack of unity of invention <input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement <input type="checkbox"/> Box No. VI Certain documents cited <input type="checkbox"/> Box No. VII Certain defects in the international application <input type="checkbox"/> Box No. VIII Certain observations on the international application

Date of submission of the demand 18.01.2005	Date of completion of this report 28.06.2005
Name and mailing address of the International preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer van de Kamp, M Telephone No. +31 70 340-2373



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Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
 - This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
 - international search (under Rules 12.3 and 23.1(b))
 - publication of the international application (under Rule 12.4)
 - international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements*** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

Description, Pages

1-41 as originally filed

Claims, Numbers

9-41 as originally filed
1-8, 42-46 received on 20.01.2005 with letter of 18.01.2005

Drawings, Sheets

1/6-6/6 as originally filed

- a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. The amendments have resulted in the cancellation of:
 - the description, pages
 - the claims, Nos. 1-8 and 42-47 as originally filed
 - the drawings, sheets/figs
 - the sequence listing (*specify*):
 - any table(s) related to sequence listing (*specify*):
4. This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
 - the description, pages
 - the claims, Nos.
 - the drawings, sheets/figs
 - the sequence listing (*specify*):
 - any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of these sheets may be marked "superseded."

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-46
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-46
Industrial applicability (IA)	Yes:	Claims	1-46
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement (Continuation)

2.1 CITATIONS

The following documents may be referred to in this report:

- D1** WO 99/13098 A (NICOLA MAZIN ; ADVANCED PHYTONICS LTD (GB)) 18 March 1999
- D2** WO 98/42687 A (DU PONT MERCK PHARMA) 1 October 1998
- D3** BROOS J ET AL: "Activity and enantioselectivity of serine proteases in transesterification reactions in organic media", JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS 1: ORGANIC AND BIO-ORGANIC CHEMISTRY, vol. 22, 1995, pages 2899-2905
- D4** WO 97/22712 A (HOECHST MARION ROUSSEL INC) 26 June 1997
- D5** KAWASHIRO K ET AL: "Effect of organic solvents on enantioselectivity of protease catalysis", BIOTECHNOLOGY AND BIOENGINEERING, vol. 53, no. 1, 5 January 1997, pages 26-31
- D6** NISHIO T ET AL: "Production of optically active esters and alcohols from racemic alcohols by lipase-catalyzed stereoselective transesterification in non-aqueous reaction system", JOURNAL OF BIOCHEMISTRY, vol. 105, no. 4, 1989, pages 510-512
- D7** WEBER H K ET AL: "'Watching' lipase-catalyzed acylations using ¹H NMR: competing hydrolysis of vinyl acetate in dry organic solvents", TETRAHEDRON: ASYMMETRY, vol. 10, no. 14, 16 July 1999, pages 2635-2638
- D8** LEMKE K ET AL: "Lipase-catalysed Kinetic Resolution of Phenylethan-1,2-diol by Sequential Transesterification - the Influence of the Solvent", TETRAHEDRON: ASYMMETRY, vol. 7, no. 4, 1 April 1996, pages 971-974
- D9** LLOYD R C ET AL: "Probing the specificity of the S1', leaving group, site of subtilisin Bacillus latus using an enzyme-catalyzed transesterification reaction", TETRAHEDRON: ASYMMETRY, vol. 9, no. 4, 27 February 1998, pages 551-561
- D10** THEIL F ET AL: "Lipase-catalyzed transesterification of meso-cyclopentane

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- diols", TETRAHEDRON, vol. 47, no. 36, 1991, pages 7569-7582
- D11** GHORPADE S R ET AL: "Desymmetrization of meso-cyclopenten-cis-1,4-diol to 4-(R)-hydroxycyclopent-2-en-1-(S)-acetate by irreversible transesterification using Chirazyme", TETRAHEDRON: ASYMMETRY, vol. 10, no. 5, 12 March 1999, pages 891-899
- D12** JOHNSON C R ET AL: "Enzymatic asymmetric resolution of meso-2-cycloalken-1,4-diols and their diacetates in organic and aqueous media", TETRAHEDRON LETTERS, vol. 33, no. 48, 1992, pages 7287-7290
- D13** GILL J ET AL: "Enantioselectivity of lipase-catalysed transesterification of 2-ethyl-1,3-propanediol: comparison of lipases from bacterial, fungal and animal sources", TETRAHEDRON: ASYMMETRY, vol. 8, no. 13, 10 July 1997, pages 2227-2230
- D14** THEIL F ET AL: "Kinetic resolution of acyclic 1,2-diols using a sequential lipase-catalyzed transesterification in organic solvents" JOURNAL OF ORGANIC CHEMISTRY, vol. 59, no. 2, 1994, pages 388-393

D10, D11 and D12 were cited in the application.

2.2 AMENDMENTS (Art. 19 PCT)

- 2.2.1** The amendments filed with the International Bureau under Article 19(1) do not introduce subject-matter which extends beyond the content of the application as filed, as the amendments are based on claims 1 and 45 as originally filed.

2.3 NOVELTY (Art. 33(2) PCT)

- 2.3.1** The following documents are regarded as being the closest prior art to the subject-matter of the independent **claims 1, 8, 19 and 30**, as follows:
- 2.3.2** **D1** discloses the use of 1,1,1,2-tetrafluoroethane (Phytosol) as a first solvent, possibly with dimethylether as co-solvent, and with various ratios of said first solvent to that of water, in the hydrolysis (deacylation) of penicillin G yielding stereochemically pure 6-APA, also applicable to protease- and lipase-catalysed reactions (cf. scheme on page 2, page 5 line 5-13, page 5 line 24 - page 6 line

18, page 8 line 14 - page 9 line 7, page 12 lines 27-29, examples 1-7, page 22 line 33 - page 23 line 1, and claims 1-13 and 17). The solvent mixture consists of two immiscible phases or layers (e.g., examples 1(iii)-(v), 3, 6) which make the method different from the one claimed in current independent **claim 1**, in particular where it refers to 'A process ... which is conducted in the presence of water at a level which is less than that required for the water to form a separate aqueous phase in the reaction system'.

- 2.3.3** Each of **D3 to D8** discloses a process of resolving a racemic mixture involving the use of a biological catalyst, but they differ from the subject matter of current independent **claim 8** in that as a solvent no (hydro)fluorocarbon is used.
- 2.3.4** Each of **D10 to D12** discloses a process of preparing a particular enantiomer from a meso compound involving the use of a biological catalyst, but they differ from the subject-matter of current independent **claim 19** in that as a solvent no (hydro)fluorocarbon is used.
- 2.3.5** Each of **D13 and D14** discloses a process of preparing a particular enantiomer from a prochiral compound involving the use of a biological catalyst, but they differ from the subject-matter of current independent **claim 30** in that as a solvent no (hydro)fluorocarbon is used.
- 2.3.6** The subject-matter of independent **claims 1, 8, 19 and 30** as well as dependent **claims 2-7, 9-18, 20-29, and 31-46** is therefore considered as new in respect of the prior art as defined in the regulations (Article 33(2), Rule 64(1)-(3) PCT).

2.4 INVENTIVE STEP (Art. 33(3) PCT)

2.4.1 *Claims 1-4, 7 and 39-46*

D1 is regarded as the closest prior art to the subject-matter of **claim 1**. It discloses the use of the (hydro)fluorocarbon compound 1,1,1,2-tetrafluoroethane (Phytosol) as a first solvent, possibly with dimethylether as co-solvent, and with various ratios of said first solvent to that of water, in the hydrolysis (deacylation) of penicillin G yielding stereochemically

pure 6-APA, also applicable to protease- and lipase-catalysed reactions (cf. scheme on page 2, page 5 line 5-13, page 5 line 24 - page 6 line 18, page 8 line 14 - page 9 line 7, page 12 lines 27-29, examples 1-7, page 22 line 33 - page 23 line 1, and claims 1-13 and 17). D1 (e.g. on page 22) contains an extensive list of advantages of the use of 1,1,1,2-tetrafluoroethane, including that a more efficient enzyme reaction is achieved involving a faster reaction and/or improved yield, that the activity of the enzyme is not damaged by said solvent system, and that the process is faster and cheaper than established industrial processes. Although in the *examples* (e.g., examples 1(iii)-(v), 3, 6) solvent mixtures are used apparently consisting of two immiscible phases or layers, which make the method different from the one claimed in current independent **claim 1**, in particular where it refers to 'A process ... which is conducted in the presence of water at a level which is less than that required for the water to form a separate aqueous phase in the reaction system', the *description* mentions high ratios of said first solvent to that of water (page 5 line 5-13). At these more extreme ratios it is unlikely that phase separation between 1,1,1,2-tetrafluoroethane and water takes place. This renders the solution as claimed in current independent **claim 1** not inventive, as it represents a possibility encompassed by the possible ratios of (hydro)fluorocarbon solvent and water as disclosed by D1, without offering further advantages which are not referred to in D1.

The subject-matter of dependent **claims 2-4, 7 and 39-46** is all comprised in D1 (cf. passages cited above). Hence the subject-matter of **claims 1-4, 7 and 39-46** is not considered as involving an inventive step, contrary to Article 33(3) PCT.

2.4.2 Claims 5 and 6

In view of the disclosure by D1 (*vide supra*), the subject-matter of the dependent **claims 5 and 6** is regarded as lacking an inventive step, because the use of enzymes as part of a whole cell culture (**claim 5**), although not disclosed in D1, is a well-known alternative way of carrying out an enzymatic reaction, and because, although abzymes (**claim 6**) are not referred to in the list of enzymes on page 22 line 35 - page 23 line 1, their activity falls within the activities of the mentioned enzymes.

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2.4.3 *Claims 8-18*

Each of **D3** to **D8** can independently be regarded as being the closest prior art to the subject-matter of **claim 8**.

D3 discloses enantioselective transesterification of N-acetyl-D,L-phenylalanine ester with 1-propanol by subtilisin Carlsberg in cyclohexane (abstract, page 2901 left-hand column line 4-10).

D4 discloses enzymatic resolution of N-acetyl-D,L-4-cyanophenylalanine ethyl ester through hydrolysis by subtilisin Carlsberg in acetonitrile/water (example 2).

D5 discloses enantioselective transesterification of N-trifluoroacetyl-D,L-phenylalanine 2,2,2-trifluoroethyl ester with 1-propanol by subtilisin Carlsberg in various organic solvents (abstract, table III).

D6 discloses stereoselective transesterification of *R* and *S* 1-phenylethanol with vinyl acetate by various lipases in various organic solvents (abstract, tables I and II, figure 3).

D7 discloses transesterification of 1-phenylethanol with vinyl acetate by various lipases in benzene-*d*₆(table 1).

D8 discloses resolution of a racemic mixture of (*RS*)-phenylethan-1,2-diol with vinyl acetate and various lipases in various solvents (table 1).

2.4.4 The subject-matter of **claim 8** differs in that as a solvent at least one (hydro)fluorocarbon is applied, resulting in a good or improved performance in terms of reaction rate, product conversion and stereo- or enantioselectivity (cf., e.g., examples 1 and 2).

2.4.5 The problem to be solved by the subject-matter of **claim 8** may therefore be regarded as to provide an alternative or improved enzymatic process for resolving a racemic mixture. The solution as proposed in **claim 8** of the present application is the use of a (hydro)fluorocarbon as a solvent in such a process.

2.4.6 This solution cannot however be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

D1 discloses the use of 1,1,1,2-tetrafluoroethane as the organic solvent in an enzymatic reaction has several advantages, not restricted to penicillin and

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cephalosporin splitting enzymes but relevant to other related enzymes including acylase, amidases, proteases and esterase. Amongst those advantages are listed (e.g., on page 22):

- a more efficient enzyme reaction is achieved involving a faster reaction and/or improved yield,
- the activity of the enzyme is not or less damaged,
- overall the process is faster and cheaper.

The skilled person, confronted with the problem posed, would therefore consider the use of a (hydro)fluorocarbon solvent in a process as said as the mere selection from several known options from which he would choose, offering the same technical effects and advantages as disclosed in **D1**.

2.4.7 Dependent **claims 9-18** do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, as the relevant subject-matter is either disclosed in the cited prior art or falls within the (general) knowledge and ability of the skilled person.

In particular, **D3** and **D4** are relevant to the subject-matter of **claims 9-12 and 14-18** (enantioselective transesterification of N-acetyl-D,L-phenylalanine esters by subtilisin Carlsberg); **D9** (table 1) discloses the suitability of methanol in the transesterification of N-acetyl-L-phenylalanine (present **claim 12**); **D5** is relevant to the subject-matter of **claims 9-12 and 14-18** (enantioselective transesterification of N-trifluoroacetyl-D,L-phenylalanine ester by subtilisin Carlsberg); and **D6** and **D7** are relevant to the subject-matter of **claims 9, 10, 13-16** (stereoselective transesterification of *R* and *S* 1-phenylethanol with vinyl acetate by various lipases).

2.4.8 *Claims 19-29*

Each of **D10** to **D12** can independently be regarded as being the closest prior art to the subject-matter of **claim 19**.

D10 discloses stereoselective transesterification of meso-cis-4-cyclopenten-1,3-diol with vinyl acetate by various lipases, e.g., Amano PS (= *Pseudomonas cepacia*) lipase, in THF/Et₃N (abstract, table 1).

D11 discloses stereoselective transesterification of meso-cis-4-cyclopenten-1,3-diol with vinyl acetate by *Mucor meihei* lipase, in various solvents, e.g., with Et₃N) (abstract, tables 1 and 6).

D12 discloses stereoselective transesterification of meso-cis-4-cyclopenten-1,3-diol with vinyl acetate by various lipases, e.g., *Candida antarctica* B lipase, in various solvents (abstract, scheme 1, table 1).

- 2.4.9** The subject-matter of **claim 19** differs in that as a solvent at least one (hydro)fluorocarbon is applied, resulting in a good or improved performance in terms of reaction rate, product conversion and stereo- or enantioselectivity (cf., e.g., examples 3, 4, 6 and 7).
- 2.4.10** The problem to be solved by the subject-matter of **claim 19** may therefore be regarded as to provide an alternative or improved enzymatic process for the selective or preferred preparation of a particular enantiomer from a *meso* compound. The solution as proposed in **claim 19** of the present application is the use of a (hydro)fluorocarbon as a solvent in such a process.
- 2.4.11** This solution cannot however be considered as involving an inventive step (Article 33(3) PCT) for the same reasons as in **2.4.6** (*vide supra*).
- 2.4.12** Dependent **claims 20-29** do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, as the relevant subject-matter is either disclosed in the cited prior art or falls within the (general) knowledge and ability of the skilled person.

In particular, **D10** is relevant to the subject-matter of **claims 20-29** (stereoselective transesterification of meso-cis-4-cyclopenten-1,3-diol with vinyl acetate by various lipases, e.g., Amano PS (= *Pseudomonas cepacia*) lipase, in THF/Et₃N); **D11** is relevant to the subject-matter of **claims 20-28** (stereoselective transesterification of meso-cis-4-cyclopenten-1,3-diol with vinyl acetate by *Mucor meihei* lipase, in various solvents, e.g., with Et₃N); and **D12** is relevant to the subject-matter of **claims 19-22 and 25-29** (stereoselective

transesterification of meso-cis-4-cyclopenten-1,3-diol with vinyl acetate by various lipases, e.g., *Candida antarctica* B lipase).

2.4.13 *Claims 30-38*

Each of **D13** and **D14** can independently be regarded as being the closest prior art to the subject-matter of **claim 30**.

D13 discloses stereoselective transesterification of 2-ethyl-1,3-propanediol with vinyl acetate by various lipases (abstract, table 1).

D14 discloses stereoselective transesterification of acyclic prochiral 1,2-diols by various lipases, e.g., Amano PS (*P. cepacia*) lipase, in various solvents (abstract, tables 1-10).

2.4.14 The subject-matter of **claim 30** differs in that as a solvent at least one (hydro)fluorocarbon is applied, resulting in a good or improved performance in terms of reaction rate, product conversion and stereo- or enantioselectivity (cf., e.g., example 5).

2.4.15 The problem to be solved by the subject-matter of **claim 30** may therefore be regarded as to provide an alternative or improved enzymatic process for the selective or preferred preparation of a particular enantiomer from a prochiral compound. The solution as proposed in **claim 30** of the present application is the use of a (hydro)fluorocarbon as a solvent in such a process.

2.4.16 This solution cannot however be considered as involving an inventive step (Article 33(3) PCT) for the same reasons as in **2.4.6** (*vide supra*).

2.4.17 Dependent **claims 31-38** do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, as the relevant subject-matter is either disclosed in the cited prior art or falls within the (general) knowledge and ability of the skilled person.

In particular, **D13** is relevant to the subject-matter of **claims 31-37** (stereoselective transesterification of 2-ethyl-1,3-propanediol with vinyl acetate

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by various lipases); and D14 (e.g., table 3) discloses the suitability of Amano PS (*P. cepacia*) lipase in this type of reactions (present **claim 38**).

2.4.18 In summary: The present application does not satisfy the criterion set forth in Article 33(3) PCT and the subject-matter of **claims 1-46** does not involve an inventive step (Rule 65(1)(2) PCT).

2.5 INDUSTRIAL APPLICABILITY (Art. 33(4) PCT)

2.5.1 The subject-matter of **claims 1-46** satisfies the criterion set forth in Art. 33(4) PCT in conjunction with Rule 5(vi) PCT with respect to industrial applicability.

Claims

1. A process for preparing a second compound stereo-selectively which
5 process comprises reacting a substrate comprising at least one first
compound with a reagent in the presence of a biological catalyst and a
solvent comprising at least one (hydro) fluorocarbon which is conducted in
the presence of water at a level which is less than that required for the water
to form a separate aqueous phase in the reaction system.

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2. A process as claimed in claim 1, wherein the biological catalyst is an
enzyme.

3. A process as claimed in claim 2, wherein the enzyme is a hydrolase.

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4. A process as claimed in claim 3, wherein the enzyme is selected
from the proteases and lipases.

5. A process as claimed in any one of claims 2 to 4, wherein the

20 enzyme is part of a whole cell culture.

6. A process as claimed in claim 1, wherein the biological catalyst is an
abzyme.

25 7. A process as claimed in any one of the preceding claims, wherein the
substrate is reacted to form an enantiomer at an enantiomeric excess of
greater than 50%.

30 8. A process of resolving a racemic mixture which process comprises
reacting that mixture with a reagent in the presence of a biological catalyst
and a solvent comprising at least one (hydro) fluorocarbon so as to

42. A process as claimed in any one of the preceding claims, wherein the at least one (hydro) fluorocarbon is used in combination with a co-solvent.

5 43. A process as claimed in claim 42, wherein the co-solvent is halogen free.

44. A process as claimed in any one of the preceding claims, wherein the solvent is in the liquid state.

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45. A process as claimed in any one of the preceding claims wherein the amount of water that is used is below the saturation level for the solvent.

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46. A process as claimed in any one of the preceding claims wherein the amount of water that is used is less than 1% by weight of water based on the total weight of the solvent.